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Note

Direct coupling of fused silica columns to the ion source of a mass spectrometer applied to studies of arachidonic acid metabolism in human fibroblasts

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The metabolites of arachidonic acid are very labile polyunsaturated hydroxy fatty acids. When studying these compounds, problems of stability during gas chromatography—mass spectrometry (GC—MS) are to be expected. It is well known that the lower detection limit of prostaglandins is relatively high, owing not only to a disadvantageous fragmentation pattern, but also to losses during GC. Resorption and destruction phenomena have to be avoided to obtain reliable spectra. The interface and the transfer line between the GC column and the ion source of the mass spectrometer may be a source of leaks and also cause destruction of substances on active centers. Once precipitated, the residues will never be removed but influence the background. These reasons prompted us to introduce the thin, unbreakable and flexible, fused-silica column directly through the interface into the ion source of the mass spectrometer, thereby avoiding [1] the problems described above. Theoretical considerations, however, indicate that the separation quality of the GC column could be influenced by the high vacuum.

INSTRUMENTATION

A Finnigan GC 9610 coupled to a Finnigan 4000 mass spectrometer with a positive electron-impact ionisation (EI) and a positive chemical ionisation (CI) device combined with an Incos data system were used for the experiments. Fused silica columns, 30 m × 0.25 mm I.D., coated with the chemical bonded phase DB-1 (equivalent to the methyl-polysiloxane SE-30) were purchased from J & W Scientific Inc. (Rancho Cordova, CA, U.S.A.). The injector and the connection to the ion source were kept at a temperature of 260°C. The GC column was kept at 100°C for 1 min after injection, and then programmed to rise to 300°C. The EI mass spectra were obtained with an electron energy of 70 eV and an emission current of 0.2 A. The CI mass spectra

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were obtained with ammonia as CI gas, an electron energy of 120 eV, and an emission current of 0.1 A. The ionizer temperature was 280°C in both cases. The scan time was chosen to be 1 sec for a mass range of m/z 100—700 (mass per electron).

INSTRUMENTAL BEHAVIOUR

The high vacuum measured with the ion gauge in the mass spectrometer was about 1.6×10^{-4} Pa when the glass capillary column was connected in the usual manner to the mass spectrometer. If a fused-silica column was directly introduced, the pressure in the mass spectrometer remained unchanged or even decreased a bit. This can be explained by the smaller internal diameter of the quartz column as compared to the glass column. When the capillary column is disconnected from the interface, the small internal diameter of the glass interface causes the mass spectrometer pressure to be maintained at 6.66×10^{-3} Pa. A 50-cm long fused-silica column directly introduced results in the same pressure level. These results indicated the probability that the direct insertion of the fused silica column into the mass spectrometer would not pose greater problems or influence the separation quality of the GC column. To test this assumption, we connected the fused-silica column first through the normal interface, and then without interface. Retention times and theoretical plates of a column coated with DB-1 were measured under both conditions for the prostaglandin (PG) 6-oxo PG F1α. The temperature program was the following: 1 min at 100°C, then increasing at 40°C/min to 200°C, then at 3°C/min to 300°C. The retention times were calculated under both conditions to be 1522 sec, reproducible at ± 2 sec. The peak widths were also identical, with ten scans at half the peak height, the scan time being 858 msec. The number of theoretical plates was 170,600 under both conditions, if calculated according to the formula of Cremer

$$n = \left(\frac{t_R}{w}\right)^2 \times 5.54$$

where n = number of theoretical plates, t_R = retention time, and w = width at half the peak height. These measurements were made on the fragments 508 and 598 in the EI mode. We then measured the fragment 540 in the CI mode with ammonia as CI gas. Thereby the pressure in the ion source was increased to 46.7 Pa, from less than 1.33 Pa in the EI mode. The retention time was not affected by this pressure increase on the end of the capillary column. So far, no side-effects could be detected; however, we found that the ion source had to be cleaned more often when a new column was used, than when using an old one. This problem could be overcome by heating the new column overnight at 320°C in the GC oven before inserting it into the mass spectrometer for the first time.

EXPERIMENTS WITH LIPOXYGENASE PRODUCTS FROM CELL INCUBATIONS

To study the GC and the MS behaviour of the different metabolites of arachidonic acid we isolated these substances from platelet incubations ac-

cording to the methods of Hamberg and Samuelsson [2] and Nugteren [3], as well as from incubations with polymorphonuclear leucocytes (PMNL) following the procedure of Borgeat and Samuelsson [4]. Thereafter, we started our own experiments with fibroblast cultures.

EXPERIMENTS WITH FIBROBLAST CULTURES

Other groups [5—8] incubated fibroblast cultures with radioactively labelled arachidonic acid and measured the metabolites by radio-thin-layer chromatography (TLC) or they used cold incubations and detected the prostaglandins by radioimmunoassay. We used GC—MS for the detection of the hydroxy fatty acids.

Fibroblasts for culture were obtained from healthy humans. For the present study, two (500 cm^2) roller flasks, each containing about 2×10^7 fibroblasts in a monolayer, were used. After washing three times with physiological saline, the fibroblasts were incubated at 37°C and 3 rpm for 60 min in 15 ml of Dulbecco minimal essential medium (DMEM; Serva, Heidelberg, G.F.R.) containing 250 μg of arachidonic acid, 0.01% albumin, and 75 μg of ionophor A 23187 [8]. Thereafter, the metabolism was stopped with 10 ml of absolute ethanol, followed by extraction as described [9]. A preseparation was carried out by TLC on silica gel (Merck Kieselgel 60) plates (Merck, Darmstadt, G.F.R.), with the solvent system diethyl ether—hexane—acetic acid (60:40:1). Zones with R_F values of 0.06, 0.39 and 0.59 were scraped off and eluted. In order to increase the reliability of our results one part of the eluate was directly derivatised for GC—MS, the other part was hydrogenated prior to further derivatisation with platinum dioxide and hydrogen in methanol [10]. Thereafter, methylester-silylether or methylester-methyloxime-silylether derivatives were formed and analysed by GC—MS. Each substance was therefore detected twice, once in its originally present unsaturated form and further fully saturated, and each form was identified by retention time as well as by mass spectra in EI mode and in CI mode with ammonia as reactant gas. We found that the saturated hydroxy fatty acids gave a better separation on the GC column and further that identification is sometimes easier, as the saturated form gives more intense fragments in CI with ammonia than the unsaturated form. Fig. 1 shows the spectrum of 12-hydroxyeicosanoic acid and that of 12-hydroxyeicosatetraenoic acid in CI with ammonia.

RESULTS

Five to 60% of the arachidonic acid incubated with the fibroblasts are converted into prostaglandin E2 (PG E2). Small amounts of 12-hydroxyheptadecatrienoic acid (HHT) could be verified in the eluate from the TLC zone containing also the different eicosatetraenoic acids (HETE). Formation of HETE was presumed on the basis of radio-TLC investigations carried out by incubation of fibroblasts with radioactively labelled arachidonic acid. With our ongoing study we found that arachidonic acid is converted by photooxidation into various HETEs; the mass chromatogram (Fig. 2) shows the presence of 5-, 9-, 11-, 12-, 14-, and 15-HETE obtained from arachidonic acid incubations; the formation of 5,12-di-HETE is also presumed.

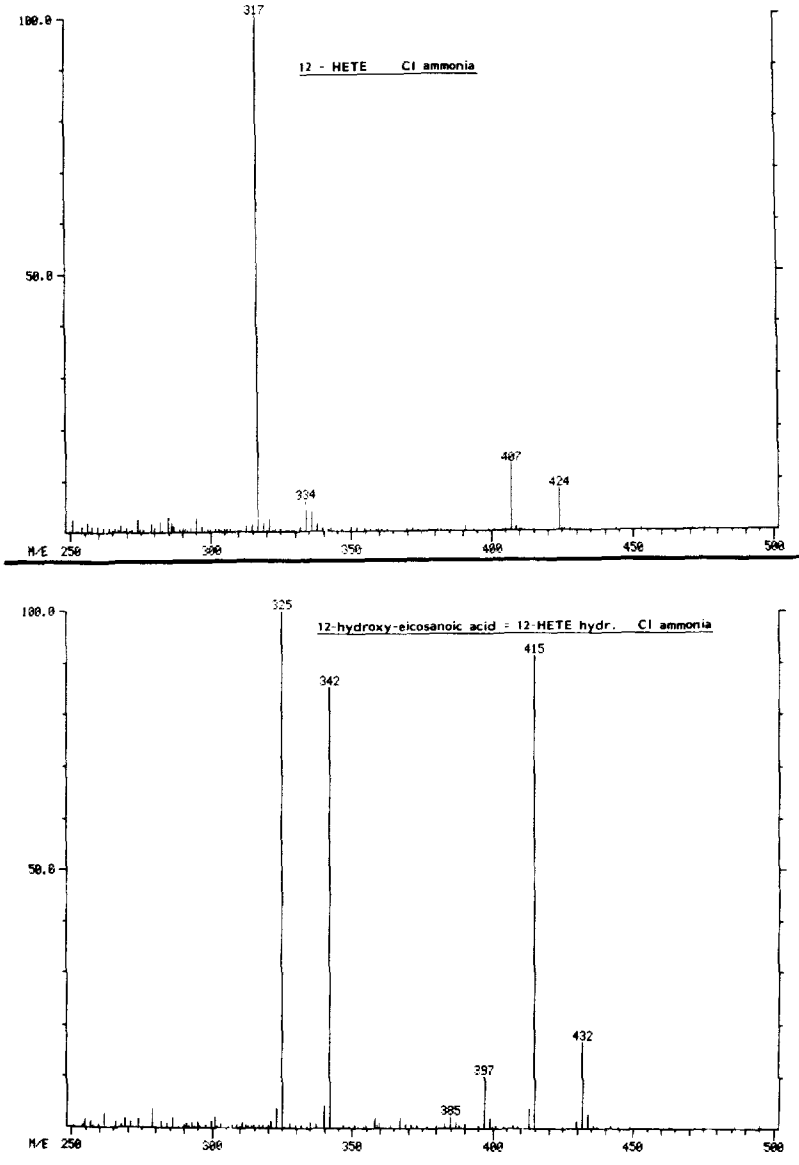

Fig. 1. Mass spectra of 12-HETE and its hydrogenated product (12-hydroxyeicosanoic acid) as methylester-silylether derivative, obtained with chemical ionisation using ammonia as CI gas.

DISCUSSION

The present study shows that the direct insertion of a fused-silica column into the ion source of a mass spectrometer is advantageous, the substances only being in contact with the coated wall of the GC column. With the present study we were able to prove that neither retention times nor number of theoretical plates are affected by this mode of coupling a GC column to the mass

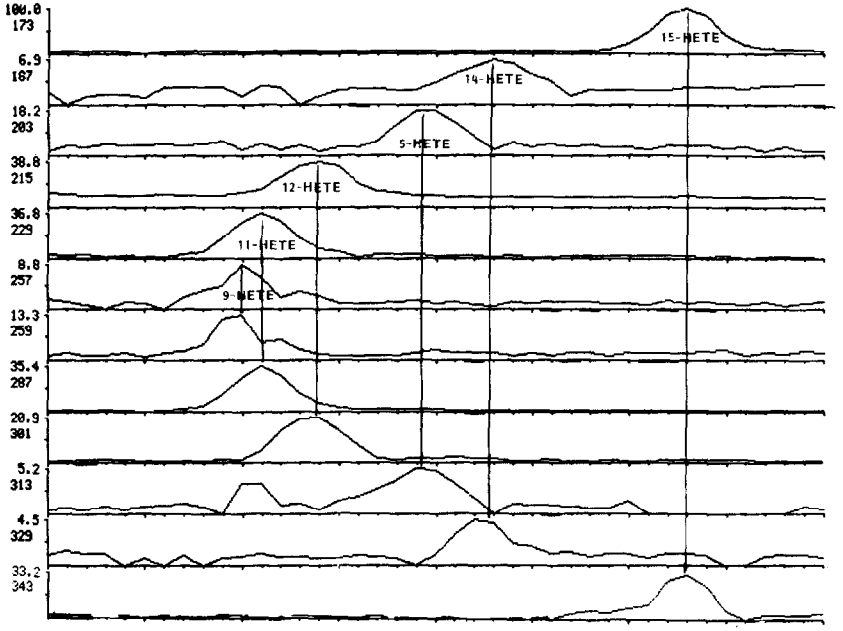

Fig. 2. Mass chromatogram of 5-HETE, 9-HETE, 11-HETE, 12-HETE, 14-HETE and 15-HETE in hydrogenated form as methylester-silylether derivatives in EI mode. The two main fragments of each substance, resulting from α-fragmentation, were monitored. The extract analysed was obtained from culture medium incubated with arachidonic acid and possibly demonstrates autooxidation.

spectrometer, which we therefore recommend. The validity of the method is demonstrated by analysing very labile arachidonic acid metabolites obtained from cell incubations. The identification of the different substances was carried out by comparison of the spectra with those obtained from substances isolated from incubations of arachidonic acid with platelets or with leucocytes according to the investigations of Hamberg and Samuelsson [2], Nugteren [3] and Borgeat and Samuelsson [4], as well as by comparison with published spectra. The two main fragments of the saturated hydroxy fatty acids result from α-fragmentation and help to differentiate the different HETEs since they are poorly separated by GC (Fig. 2).

The application of GC—MS enabled us to identify with high accuracy six different forms of HETE, namely 5-, 9-, 11-, 12-, 14-, and 15-HETE, by retention times of the natural product as well as of its saturated form and in both forms by MS in EI and CI modes. So far, identification of the different HETEs extracted from cell incubations would not pose further problems. The observation of a relatively rapid photo- or autooxidation of arachidonic acid into HETE but not into HHT suggests a very careful judgement of findings made on the enzymatic formation of HETE. We hope that we can precisely state if and which HETE is formed by fibroblast cultures. The widely varying amounts of PG E2 can probably be explained by the fact that prostaglandin formation is in close relation to the state of growth of the fibroblasts, as described by Taylor and Polgar [11].

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REFERENCES

1 H. Gleispach, E. Wurz and B. Mayer, in S. Görög (Editor), Advances in Steroid Analysis, Akadémiai Kiadó, Budapest and Elsevier Scientific Publishing Company, Amsterdam, New York, 1981, p. 307.
2 M. Hamberg and B. Samuelsson, Proc. Nat. Acad. Sci. U.S., 71 (1974) 3400.
3 D.H. Nugteren, Biochim. Biophys. Acta, 380 (1975) 299.
4 P. Borgeat and B. Samuelsson, Proc. Nat. Acad. Sci. U.S., 76 (1979) 2148.
5 N.K. Hopkins, F.F. Sun and R.R. Gorman, Biochem. Biophys. Res. Commun., 85 (1978) 827.
6 K.A. Chandrabose, E.G. Lapetina, C.J. Schmitges, M.I. Siegel and P. Cuatrecasas, Proc. Nat. Acad. Sci. U.S., 75 (1978) 214.
7 A.E. Ali, J.C. Barrett and T.E. Eling, Prostaglandins, 20 (1980) 667.
8 St.J. Feinmark and J.M. Bailey, J. Biol. Chem., 257 (1982) 2816.
9 H. Gleispach, B. Mayer, L. Rauter and E. Wurz, J. Chromatogr., 273 (1983) 161—165.
10 M. Claeys, Personal communication.
11 L. Taylor and P. Polgar, Prostaglandins, 22 (1981) 723.